



Infancia y Medio Ambiente

INMA 14-16 AÑOS

ANEXO 5a: PROTOCOLO PARA EL ESTUDIO DEL SUEÑO Y ACITIVIDAD FÍSICA Y LOS DISPOSITIVOS UTILIZADOS EN LA VISITA

1. SOP Estudio sueño y actividad física

Dispositivo GENEActiv (para todos los participantes de la Cohorte)

Equipamiento y materiales

El pack de GENEActiv incluye el reloj, cargador, USB para conectar el cargador al ordenador, y un CD con el software (si no se puede descargar directamente de la web).



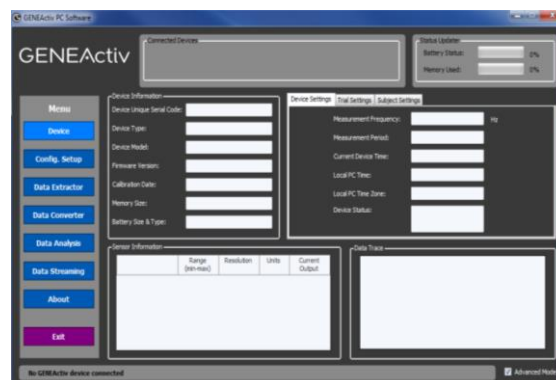
Configuración (antes de la visita)

Se instalará el programa y los drivers en los ordenadores de los trabajadores de campo. Antes de entregar el reloj al participante, este debe ser cargado y configurado.

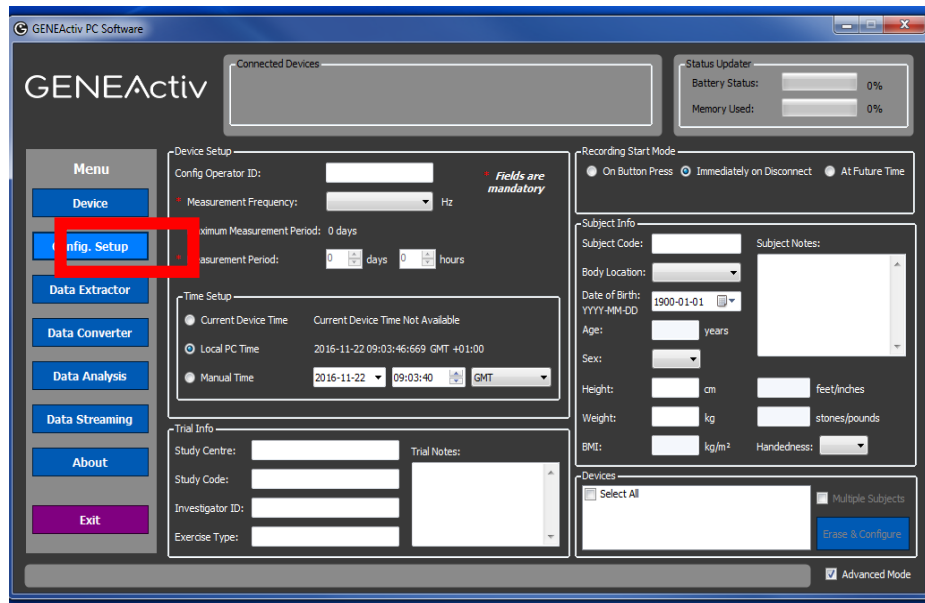
- Inserta el reloj en el cargador (y el cargador conectado al ordenador o a la corriente). Asegúrate que el reloj encaja correctamente en su lugar.
- Los relojes se deben cargar 3h antes de utilizarlos con el programa. Una luz roja intermitente aparecerá para mostrar que se está cargando. Cuando esté completamente cargado aparecerá una luz verde intermitente, entonces el reloj ya puede ser utilizado.



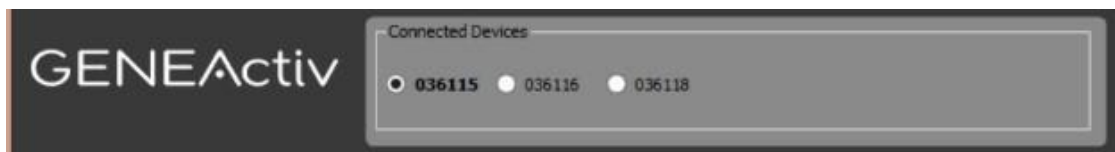
- Enciende el programa



- En el programa aparece información sobre el reloj y datos previamente almacenados.
- Clica **Config. Setup** en la parte izquierda del menú.

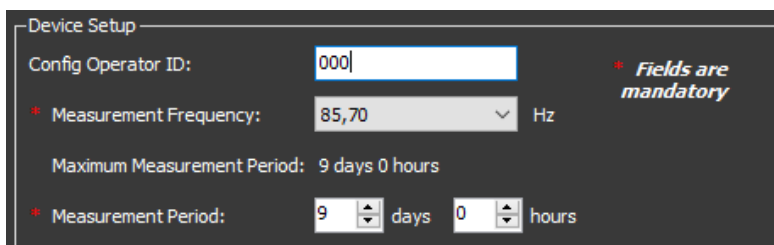


- Se pueden conectar un máximo de 4 relojes al cargador cosa que permite configurar 4 relojes a la vez. El identificador de los relojes conectados aparecerá en el cuadro de **Connected devices**

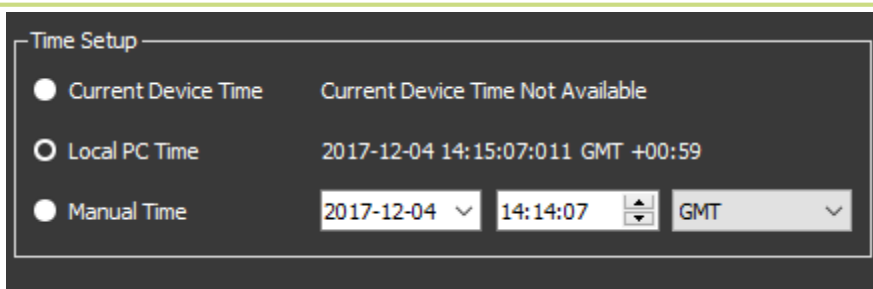


- Completa lo siguiente **PARA CADA RELOJ**:

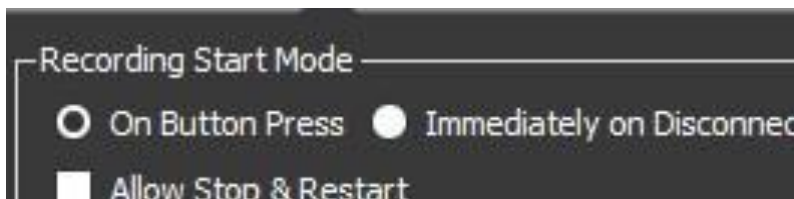
- ✓ **Config Operator ID:** introducir el id del participante
- ✓ **Measurement Frequency:** La frecuencia de medida seleccionada determinará el máximo de datos de tiempo que pueden ser almacenados (a menos frecuencia, más tiempo).
 - Configurar a 85.7Hz
- ✓ **Measurement Period:** permite seleccionar el periodo en el que se estará recogiendo datos.
 - Configurar a 9 días



- ✓ Seleccionar **Local PC Time**



- ✓ Seleccionar cuando quieres que empiece a recoger datos.
- **Configurar a On Button Press**



NOTA: Es muy importante que **Allow Stop & Restart** esté desactivado ya que esto permitiría a los participantes desactivar el reloj.

- Clica **Erase & Configure, Select all** y **Multiple Subjects** todos los relojes que estén conectados quedará configurados



Procedimiento con el participante (durante la visita)

- Colócale el reloj en la muñeca de la mano no-dominante.
- Explícale que el reloj es resistente al agua y que no es necesario que se lo quite a menos que haga un deporte de riesgo que pueda dañar a él mismo o a otros por llevarlo (ej. Karate).

Anotar en la hoja de monitorización (ver apartado formularios de registro) el código del dispositivo, la fecha del día 0 de inicio y la del día 8 de recogida

Entregar al voluntario las instrucciones en el Diario semanal de monitorización del Documento Anexo 5d_Diario_semanal_INMA (si sólo lleva este dispositivo) o el Documento Anexo 5d_Diario_semanal_ATHLETE (si lleva alguno más, detalles en el siguiente apartado)

Descargar los datos (después de la visita)

- Después de una semana, recoge el reloj y el diario y descarga los datos del reloj.
- Abre el programa y conecta el adaptador al Puerto USB.
- Inserta los relojes en el adaptador.

- Clica **Data Extractor** en el menú de la izquierda. Esta página enseña un gráfico con los primeros minutos recogidos y permite descargar los datos del reloj escogido
 - Seleccionar el reloj a descargar arriba, en **Connected devices**



- Selecciona en **Browse** la carpeta donde quieres que se guarde el archivo. Reescribe el nombre del archivo:

SabXXX_YYYYYY_DDMMAAAA.bin

XXX: numero identificativo del participante

YYYYYY: Número de serie del reloj

DDMMAA: fecha (ej:16122016, 16 de diciembre del 2016)

- Clica **Start Extract** y espera
- Haz lo mismo para todos los relojes conectados.
- Descargar los datos de cuatro relojes son 20 minutos.
- Al final de la descarga comprueba que el archivo se ha guardado en la carpeta y con el nombre correctos.
- También es importante comprobar que el tamaño del archivo es consistente con el resto de archivos. Esto sería correcto:

Sab35.bin	20/02/2017 14:02	Archivo BIN	712.614 KB
Sab115.bin	20/02/2017 14:36	Archivo BIN	827.803 KB
Sab129.bin	20/02/2017 14:31	Archivo BIN	643.823 KB

- Si detectamos que un archivo tiene un tamaño muy menor, por ejemplo, el "Sab413", se tienen que volver a descargar los datos de este reloj. Si se repite el tamaño asumimos que el reloj no tiene más datos y lo dejamos así. Si el tamaño es menor pero diferente se tiene que volver a repetir la descarga.

Sab250.bin	20/02/2017 13:59	Archivo BIN	744.739 KB
Sab376.bin	20/02/2017 14:41	Archivo BIN	827.803 KB
Sab396.bin	20/02/2017 14:04	Archivo BIN	734.251 KB
Sab413.bin	20/02/2017 14:38	Archivo BIN	4.875 KB

- Carga el reloj hasta que está completamente cargado (unas 3h).

2. OTROS DISPOSITIVOS EN LA VISITA

I. ACTIGRAPH: PERSONAL PHYSICAL ACTIVITY MONITORING USING AN ACTIGRAPH wGT3X-BT (ATHLETE) (ENG)

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Contact person: Sarah Koch

ATHLETE SOP coordinator for personal exposure monitoring: Mark Nieuwenhuijsen (mark.nieuwenhuijsen@isglobal.org)

1. PURPOSE AND APPLICABILITY

The purpose of this SOP is to describe the personal monitoring of physical activity with ActiGraph personal monitors for the Athlete study, and also to standardize the operation of ActiGraph GT3X-BT.

2. RESPONSIBILITIES AND USERS

1. The ATHLETE SOP coordinator for personal exposure monitoring is responsible for ensuring that new versions of this SOP are available for the relevant members of the project team and its instructions are properly implemented.
2. This SOP will be principally applied by ATHLETE fieldworkers.
3. If the procedures of this SOP are changed, the change has to be documented according to instructions and it has to be communicated to the SOP coordinator.
4. The users of the SOP are obliged to work according to this SOP. All potential deviations in implementing this SOP have to be carefully documented (who changed, when, why, what changes, possible impacts) and reported.

3. DEFINITIONS AND ABBREVIATIONS

FEF: Field Exposure Form for participants to report incidences during monitoring.

MS: Monitoring sheet for fieldworkers to report incidences during field monitoring.

SOP: Standard Operating Procedure.

FW: Field Worker

MET: 1 MET (Metabolic Equivalent of Task) is defined as the amount of oxygen consumed while sitting at rest and is equal to 3.5 ml O₂ per kg body weight x min. The MET concept represents a simple, practical, and easily understood procedure for expressing the energy cost of physical activities as a multiple of the resting metabolic rate.

Participant: An adolescent participating in the ATHLETE follow-up

Subject ID: Each subject is uniquely identified by an ATHELTE identification code.

4. EQUIPMENT AND MATERIALS

The ActiGraph will be worn by study subjects for seven consecutive days (one entire week) during waking hours.

4.1. Equipment

ActiGraph wGT3X-BT model

ActiLife software: <https://www.actigraphcorp.com/support/software/>.

Laptop, the same for all monitoring instruments, with internet access and laptop time synchronized to an atomic clock.

4.2. Materials

- Spibelt to attach the ActiGraph (device must be on the right-hand side of the belt)
- USB cables to connect the ActiGraph to the laptop

- Monitoring sheet for fieldworkers: form to be completed by the FWs when installing the devices and deploying them from the subjects' home.
- Incidence sheet for the participants: handed to participants to report any incidences during the sampling period.

5. PROCEDURES

5.1. GENERAL OVERVIEW

One goal of the ATHLETE study is to measure participants' personal physical activity levels by using the wGT3X-BT (Figure 1). These devices provide objective measurements of human physical activity and are used in many research and clinical environments.

5.1.1. Physical activity

The ActiGraph wGT3X-BT are the activity monitors used in the ATHLETE study to capture and record continuous, high resolution physical activity. Monitors contain a 3-axis micro-electro-mechanical system (MEMS) accelerometer with a dynamic range of +/- 8 G. The acceleration data is sampled by a 12 bit analog to digital converter at rates ranging from 30 Hz to 100 Hz (user selectable) and stored in a raw, non-filtered/accumulated format in the units of gravity (G's). This data is stored directly into non-volatile flash memory.

The wGT3X-BT device can be worn at the waist or on the non-dominant wrist using adjustable belts or wrist straps. The appropriate wear location is dependent on specific research objectives of each study. For ATHLETE, ActiGraph will be worn on the waist, sitting on top of the hipbone using a SPIbelt (figure 2).



Figure 1. ActiGraph wGT3X-BT



Figure 2. SPIbelt

5.1.2. Device waterproofness

The wGT3X-BT is water resistant on immersion in one meter of water for up to 30 minutes if the USB port is properly closed, otherwise, water can get into the device and damage the electronics. Since this study deploys the devices to many different participants, we will treat the devices as **non-waterproof** to avoid any possible damage. Participants should be advised that the devices need to be taken off and removed when showering, bathing, or swimming. Immediately after taking the device off (due to being in contact with water, for example, for showering), the devices should be put back on and worn again so data is collected.

5.1.3. Battery life and LED decoding

ActiGraph battery can last up to 25 days when wireless is disabled and with a 30Hz sampling rate. At a higher sampling rates, battery life is 19 days with a data storage capacity of 16 days of data recording. However, this depends on the previous usage for each device; therefore, it is worth testing each single device before starting the measurements. The minimal requirements for the ATHLETE study is to allow for data collection for 7 full days at a sampling frequency of 30 Hz.

The ActiGraph contains a rechargeable lithium-ion battery that should be fully charged before initialization and deployment to subjects. The ActiGraph software, ActiLife, will not initialize a

device if the battery has dropped below 3.1 volts while in use as the device will not have sufficient power to collect data and will warn the user through a series of coded flashes (Table 1 and Table 2). The battery level, reported in volts, can be viewed at any time by starting the ActiLife software and plugging the device in.

The ActiGraph's battery has a maximum voltage of approximately 4.20 volts. At 3.1 volts the devices enter a *Low Voltage Mode* state (called HALT mode); the battery discharges beyond a point of being able to power the device. In this mode, all important variables and data are stored in flash memory to secure the device download. Because the device's internal clock stops in HALT mode, the device cannot be recharged and redeployed; the device must first be downloaded and reinitialized to continue use. Please note that ActiGraph will not allow initialization if the voltage is below 3.85 volts.

Neither a PC nor any software are required to charge the device. Charging the device is automatic and is accomplished by connecting the device to a standard USB port. It takes no more than 3 hours to charge a fully depleted battery.

Charging instructions:

1. Unscrew the USB port cover by turning it counterclockwise with the triangle tool (Figure 3).
2. Connect the ActiGraph to a laptop/PC using a USB cable.
3. While charging, the ActiGraph will display a flashing green LED.
4. The device is fully charged once the green LED stays on steady, and 100% battery will be displayed on the ActiLife Software. Select 'Refresh' to update the voltage level during charging.
5. Screw the USB port properly so it is protected from water and dust.



Figure 3. Actigraph USB port to charge and download data

5.1.4. Battery Conditioning and Storage

While Lithium Ion batteries provide the highest energy density on the market, their shelf life can be negatively impacted if not properly stored during periods of no use. To slow the aging of the Lithium Ion battery, it is recommended that devices be stored in a partially charged state (40 – 60% battery capacity is ideal) in low ambient temperatures. It is important then to charge the devices even when they are not being used.

Note: If a computer is not available or if multiple devices need to be recharged, a self-powered USB hub can be used. **IMPORTANT:** Remember units must be charged fully before their use.

Table 1. Flashing codes when the device **IS** connected to a PC

RED LED (Fault Indicator)

2 Flashes	Li-Ion Battery is Faulty
3 Flashes	A hardware failure occurred while recording data. Contact customer support

GREEN LED

1 Flash	Battery Charging
Multiple Flashes	Communicating with PC via USB
Steady On	Battery Fully Charged

Table 2. Flashing code when the device is **NOT** connected to a PC

RED LED (Fault Indicator)

No Flashing (LED Off)	Normal operating condition or battery dead
2 Flashes	Low Battery (use ActiLife software to check for remaining battery life). The unit needs to be recharged.
3 Flashes	Unexpected Battery Failure (Temporary battery power loss) - or - - Battery Level has fallen below 3.1V and the unit has entered Halt Mode

GREEN LED

No Flashing (LED Off)	Actively collecting data ("Flash Mode" disabled) or battery dead
1 Flash	- Delay before start mode (the LED always flashes prior to starting data collection) - Actively taking data ("Flash Mode" enabled – not recommended)
2 Flashes	N/A
3 Flashes	- End of memory reached (Device no longer collecting data) - Battery died while unit was in delay before start mode (no data collected on device)

Note: The Red LED will ALWAYS flash to indicate LOW BATTERY regardless of whether "Flash Mode" is enabled or disabled. If a "stop time" (optional) has been reached, the Green LED will stop flashing all together regardless of its previous state.

5.1.5. Installing ActiLife software

You must be running ActiLife version 6.13.3 or higher to operate the wGT3X-BT device.

1. Go to: <http://www.actigraphcorp.com/actilife>
2. Click the 'Download' button. Follow the instructions to install the ActiLife software on your PC. When prompted, enter the ActiLife license key that was provided at the time of purchase to complete the installation. Ask the coordinator in case you need more information.

Note: The ActiLife FULL version and ActiLife LITE can both be used to initialize and download data from the wGT3X-BT. However, the ActiLife FULL version is required to view and/or process the collected data. FWs will have a LITE version, and the Coordinator will keep the FULL version.

5.2. MONITORING PROCEDURE

This section describes the procedure to properly configure and hand out the device to the participants before the monitoring week. Configuration of the device will be performed with a laptop before handing the ActiGraph to the participant.

The ActiGraph wGT3X+ is a triaxial accelerometer which can store raw acceleration data for long periods of time. The data reflect acceleration vectors with gravity as a reference point (vertical axis, -1G in Axis1). In three perpendicular axis vectors are collected and the vector sum is calculated. The latter is done on a minute-by-minute basis.

For the ATHLETE project, devices are worn on the waist, sitting just above the participant's right hipbone. Data is collected at a frequency of 30Hz.

5.2.1. Setting up the ActiGraph and starting data collection

1. Open the ActiLife software.

2. Connect the ActiGraph to the PC/laptop using the USB cable. The monitor will appear in the grid under the 'Devices' tab (Figure 4). Please check the information on the battery status. The information shown is the information recorded for the previous participant (which you will have already downloaded).

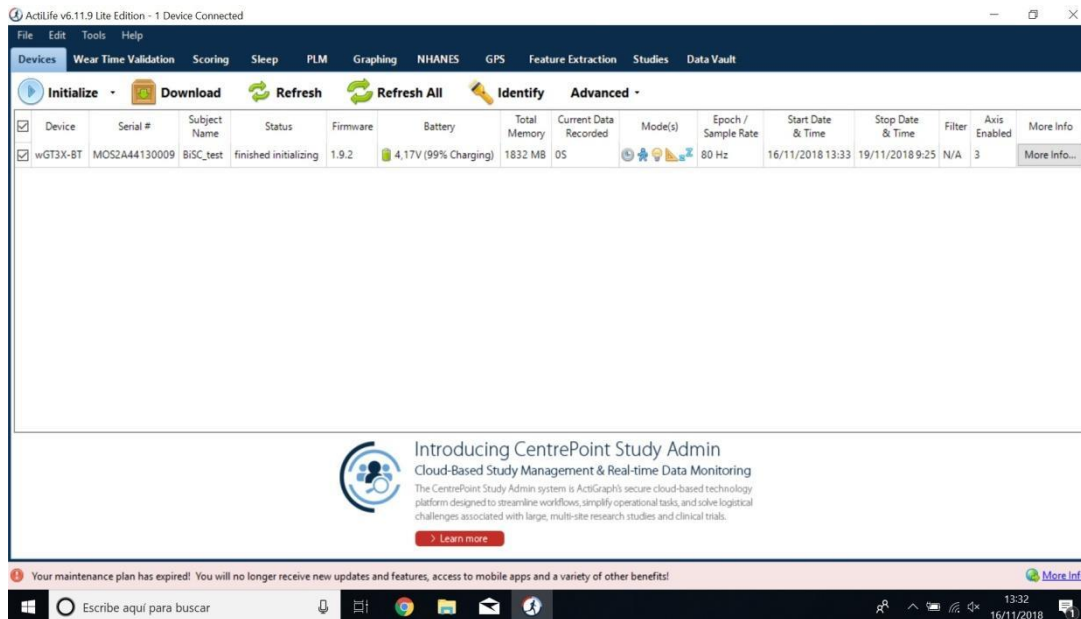


Figure 4. Overview of the ActiLife software with one ActiGraph wGT3X-BT device connected.

3. Select '**Initialize.**' A submenu will open to display several initialization options. Select '**Regular Initialization**' from the submenu. A dialog box will open to display the initialization parameters listed below (Table 3, Figure 5). The most commonly used initialization parameters are pre-selected as system defaults. However, now it's time to initialize the parameters if you choose a different option than the default.

Table 3. Parameters when configuring the device.

Parametre	Setting
Use Stop Time?	Unticked
Sample Rate	30Hz
Delay Mode LED	Unticked
Data collection LED	Unticked
Enable wireless	Unticked
Idle Sleep Mode	Disabled

For "**Select start time**", select an approximate time when the device will be fitted to the participant. "**Use stop time**" should not be selected, and the device should record until the battery is depleted, the memory capacity is full, or until the participant returns the device and data is downloaded and the device reinitialized. Check that the device time is displaying the "**Use local computer time**", which will be synchronized with an atomic clock. The laptop time needs to get an internet connection at least once a day to get updated the atomic time.

Select the device "**Sampling Rate**" at 30Hz. The sampling rate is the number of times per second that the activity monitor will record data. Note that higher sampling rates will result in higher data fidelity but will reduce the device's storage capacity and battery life. Then click on "**Enter Subject Info**".

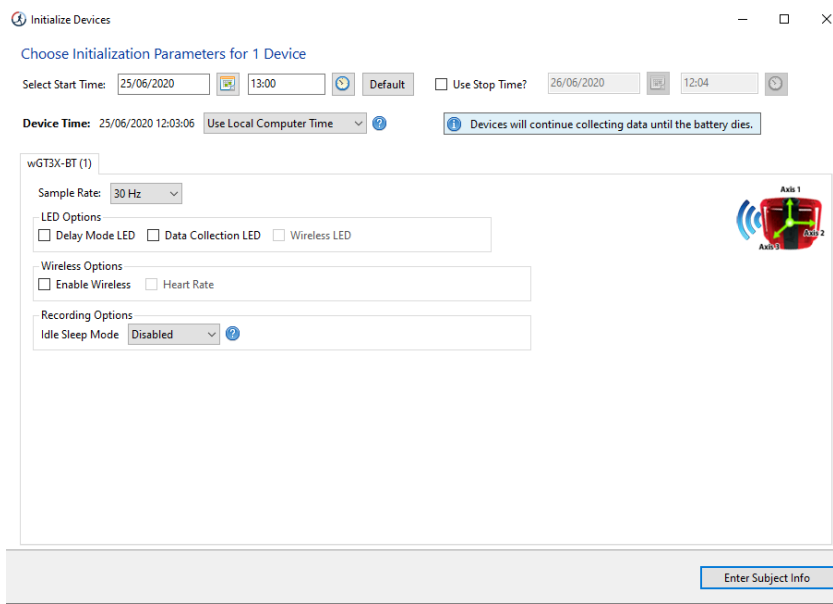


Figure 5. Initialization of the ActiGraph.

A screen with some other recording options is shown (Figure 6). Add the ATHLETE participant ID in the “**Subject info**” box, and “**Limb**” must be on the waist. If preferred, you can enter some biometric information and wear details when setting, when downloading or at data analysis. However, for ATHELTE we will not introduce any other subject information. Then select “**Initialize 1 Device**”.

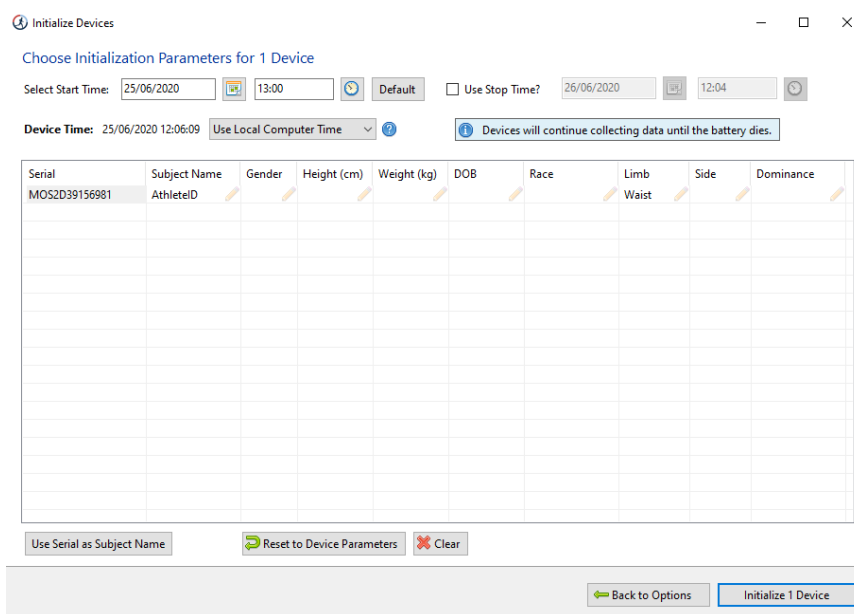


Figure 6. Recording options and subject information

After selecting “Initialize 1 Device”, a progress bar in the devices grid will indicate when the initialization process is completed. Please check the battery level again in case it needs further charging.

After the initialization is complete, unplug the ActiGraph and close the USB cap by turning it clockwise with a clip tool. Be careful not to overtighten it. The device will begin collecting data when the selected start time elapses and will continue to collect data until the device is downloaded and reinitialized.

Note: Multiple devices can be initialized simultaneously using one or more ActiGraph approved charging hubs. In this case, all devices will be programmed with the same initialization parameters and the Subject Info grid will display serial numbers of all connected devices for assignment.

5.2.2. Wearing the Device

Subjects should be instructed to wear the device strapped securely on the belt, on top of their right hipbone. The ActiGraph is waterproof ONLY when the cap is properly locked, so we will ask participants NOT TO WEAR the device while they are in contact with water, so they should remove it before showering/bathing/swimming.

Attach the SPIBelt waist belt to the participant and adjust the straps until the belt fits snugly around the waist. Position the GT3X-BT on the SPIbelt so it is facing upwards (Figure 7). The USB port cover **must** be positioned upwards/towards the participant's head. The ActiGraph wGT3X-BT must be sitting just above the participant's **RIGHT hipbone** (Figure 8). Double check that the USB port cover is facing upwards.



Figure 7. ActiGraph must be facing upwards.

The device should be worn during the day (**all waking hours**) and the belt removed each night before bed and reattached each morning just after getting out of bed or after showering/bathing. The participant should be instructed to fasten the belt snugly around the waist and position the wGT3X-BT USB port cover **facing up** on the right hipbone.



Figure 8. Location of the ActiGraph on the right hipbone on a SPIbelt.

Study participants will be given an incidence sheet to complete in case they have a problem or an incidence with the ActiGraph ("ATHLETE_FieldExposureForm_participants_incidencies" document) or any other study devices. An incidence could be *i.e.* 'swimming in the pool/sea'; taking the ActiGraph off to shower does not need to be noted on the sheet, only activities that take longer than 15min.

They will also be given a copy of any study specific instructions for wearable devices to take home (including a study specific contact phone number) with information of all the devices they will have during the measurement week, so they can check in case they have doubts on how the devices work. They will be also asked to contact FWs in case they need to.

The ActiGraphs should be cleaned after each deployment using an alcohol-based solution or wipe. SPIbelts should be handwashed.

5.3. DATA DOWNLOADING

Data will be downloaded at the end of the monitoring week, right after collecting the devices from the participant.

1. Open the ActiLife Software.
2. Connect the ActiGraph to the PC/laptop using the USB cable. The monitor will appear in the grid under the 'Devices' tab (figure 9).
3. Verify that the box of the device is checked and select '**Download**' from the taskbar menu (Figure 9).
4. A dialog box will open to display the download options listed below (Figure 10). The most commonly used download parameters are pre-selected as defaults. For ATHLETE, use the options shown in Table 4. Epoch will be set at 10 seconds.

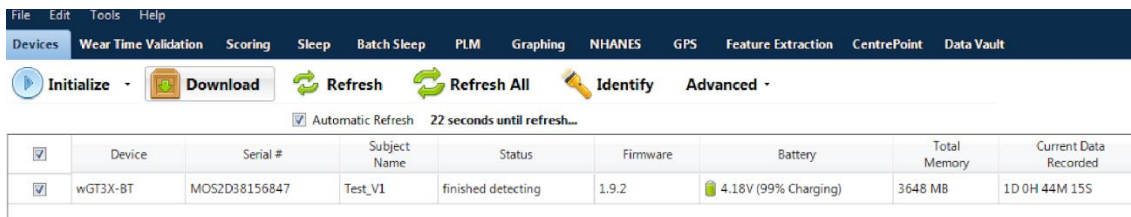


Figure 9. Once the device is connected, the software will display it and now it is ready for download.

Table 4. Downloading data parameters

Parametre	Setting
Download name convention	subject name, start date
Create Clinical Report on Download for ActiSleep Monitor(s)	Unticked
Create AGD File	Ticked
Epoch	10 seconds
# of Axis	3
Steps	Ticked
Lux	Unticked
Inclinometre	Ticked
Low Frequency Extension	Ticked
Add Biometric and user information	

Select where you want to download the files by changing the path in “Change Location”. “**Download Naming Convention**” provides a list of file name formats; choose “Subject Name, Start Date” as the subject name (the participant ID) was previously introduced. In “**Download Options**” choose “Create AGD File”. Then an AGD file, required for data scoring, will be automatically created when download.

Download Options

Change Location... C:\Users\imap\Documents\ActiGraph\ActiLife\Downloads

Use as Default Download Directory

Download Naming Convention

- <Serial Number> <Download Date>
- <Serial Number> <Start Date>
- <Subject Name> <Download Date>
- <Subject Name> <Start Date>
- Serial Number
- Subject Name
- Prompt for Each Download
- Concatenate Custom Fields

Download Options

Create Clinical Report on Download for ActiSleep Monitor(s) [edit options...](#)

Create AGD File: (wGT3X-BT)

Epoch: 10 seconds

of Axis: 3 [Select 1 sec for PLM analysis](#)

Steps Lux Inclinator Low Frequency Extension

Add biometric and user information

Download All Devices

Serial Number	Subject Name	Gender	Height (cm)	Weight (kg)	Date of Birth	Race	Limb	Side
MOS2D39156981	AthleteExpoPilot7	Female	160	60	23/04/1972 14:58		Waist	Right

Figure 10. Downloading the data using ActiLife Software.

Select the desired epoch length for the AGD file. The epoch length represents the amount of time the raw acceleration data is summed after the filter is applied. It is typically 10sec. Select which axes of data should be included in AGD file (1 Axis=Y; 2 Axis=X, Y; 3 Axis=X, Y, Z). **Select 3 axes.** Select "Steps" in order to include step count data in AGD file. Select "Inclinometer" in order to include positional data (standing, sitting, or lying down and when the device has been removed) based on device orientation in the AGD file. Select "Low Frequency Extension" to apply a low frequency filter extension to AGD file. Click on "**Download all devices**".

Note: The Low Frequency Extension option should only be used in very specific cases where physical activity is at such a low level that it might otherwise be eliminated with our normal filter. An example would be very slow shuffling movements, common in elderly populations.

A customizable PDF report containing summary data can be created with the FULL license. Subject measures and scoring algorithms can be selected by clicking 'edit options.'

Data has now successfully been downloaded from the ActiGraph and can be cleaned and scored using the analysis tools in ActiLife. The two raw data files created are **1) ID.gt3x and 2) ID10sec.agd files:**

1. ActiLife GT3X File (RAW Device Data) (.gt3x)
2. ActiLife AGD File (Accumulated Device Data every 10 seconds) (.agd)

When using the FULL license, and the download is complete, a 'finished downloading' link will appear (figure 11). Click this link to reveal options to view data, export the raw data file, and navigate to the download folder. Ensure the export settings are as show in Figure 12 then click 'Export RAW Files' to complete the export.

LITE license does not allow doing that, so you can convert the data once data collection of the study is ended (ask your coordinator for a FULL license). You can upload files and then .agd files can be exported to .csv by going to File -> import/export -> AGD -> Data Table (.CSV) (Figure 13). Save the data with the ID of the participant.

The collected data will remain on the ActiGraph device until it is reinitialized. For more information on data scoring and analysis using the ActiLife software, see the ActiLife User's Manual.

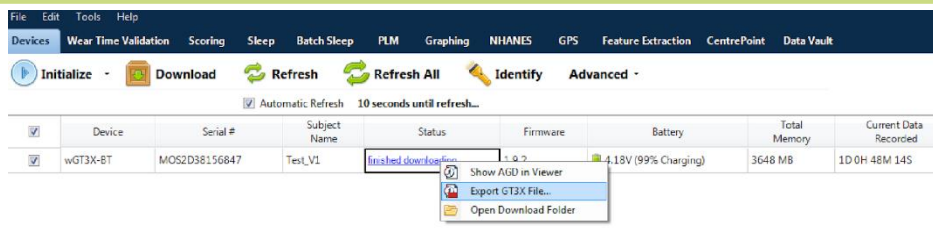


Figure 11. Exporting data with the FULL license.

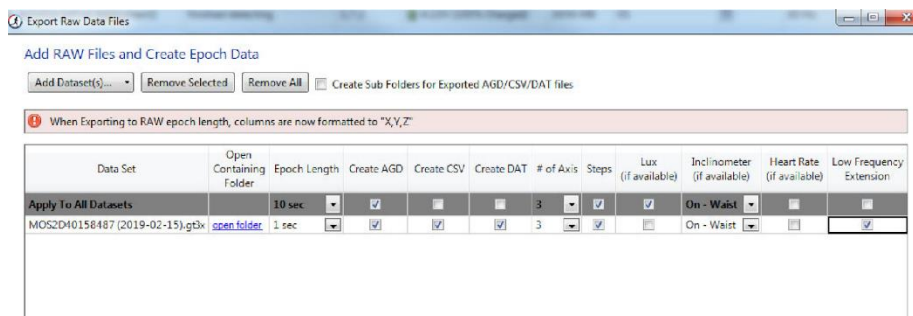


Figure 12. Creating epoch data from raw files.

Figure 13. Exporting the .agd file to a .csv file

Navigate to the participant folder, and double check the GT3X, AGD, CSV and DAT files have all been saved (this is once you have converted the files using the FULL license). The GT3X file (ActiLife GT3X File (RAW Device Data, .gt3x)) is the raw data and **MUST BE DOWNLOADED** and saved correctly to extract and analyse the data.

5.4. DATA STORAGE

Data will be always stored under the ID number of the participant. FWs will store the data first on their laptop, then copy it to an external hard drive and finally uploaded to a server.

6. QUALITY CONTROL

6.1. Physical activity validation

Physical activity data will be checked to make sure the device is reading properly, so the energy expenditure in METs will be checked to find any inconsistencies. Light intensity activities are those < 3 MET, moderate intensity activities 3-6 MET, and vigorous intensity activities > 6 MET.

6.2. Wearing time validation

Wearing time should preferably be around 16 hours per day for the whole week of measurements; however, a minimum of 10 hours is needed to have a valid day. ATHLETE will not ask participants to re-wear the devices in case this criterion is not achieved.

Data and wearing time will be checked a posteriori by the ATHLETE data manager using the statistical computing program R.

Anotar en la hoja de monitorización (ver apartado formularios de registro) el código QR del dispositivo (situado en su parte inferior), la fecha del día 0 de inicio y la del día 8 de recogida

Entregar a los voluntarios las instrucciones en el Diario semanal de monitorización (ver documento Anexo_5d_Diario_semanal_ATHLETE)

II. APLICACIÓN EN DISPOSITIVO MÓVIL EXPOAPP3

Ver SOP en el documento Annex 5c_ExpoApp3_ATH_v3.

Anotar en la hoja de monitorización (ver apartado formularios de registro) el código del móvil y el número de experimento, la fecha del día 0 de inicio y la del día 8 de recogida

Entregar a los voluntarios las instrucciones en el Diario semanal de monitorización (ver documento Anexo5c_Diario_semanal_ATHLETE)

III. NO2 PERSONAL MONITORING WITH PALMES PASSIVE DIFFUSION TUBES-ATHLETE (ENG)

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PURPOSE AND APPLICABILITY

1. PURPOSE AND APPLICABILITY

The purpose of this SOP is to describe the personal monitoring of NO₂ concentration with Palmes passive diffusion tubes for the ATHLETE study.

2. RESPONSIBILITIES AND USERS

1. The ATHLETE SOP coordinator for personal exposure monitoring is responsible for ensuring that new versions of this SOP are available for the relevant members of the project team and its instructions are properly implemented.
2. This SOP will be principally applied by ATHLETE fieldworkers.
3. If the procedures of this SOP are changed, the change has to be documented according to instructions and it has to be communicated to the SOP coordinator.
4. The users of the SOP are obliged to work according to this SOP. All potential deviations in implementing this SOP have to be carefully documented (who changed, when, why, what changes, possible impacts) and reported.

3. DEFINITIONS AND ABBREVIATIONS

- **SOP:** Standard Operating Procedure.
- **FW:** Field Worker
- **Participant:** An adolescent participating in the ATHLETE follow-up study.
- **Participant ID:** Each participant is uniquely identified by an ATHLETE identification code. (ATHLETE ID=INMA ID)
- **Diffusion tube:** type of passive sampler consisting of a tube, open at one end, with an absorbent at the other (closed) end for absorption of a specific pollutant from the surrounding air.
- **Diffusion tub ID:** Each diffusion tube is uniquely identified by an ATHLETE identification number.
- **End cap:** the plastic cap fixed on the closed end of a diffusion tube, which holds the absorbent-coated grids.
- **Grid:** used here to refer to the small mesh grids used inside the diffusion tube, which are coated with the absorbent TEA.

- **Membrane:** a permeable membrane fixed across the open end of a tube: routinely used in sulphur dioxide tubes to prevent the ingress of particulate sulphate. It has been used experimentally in nitrogen dioxide tubes to reduce interference due to wind effects.
- **Mesh:** used here to indicate a mesh fixed across the open end of a diffusion tube, used experimentally to minimize interference from wind effects (the term “mesh” is used to distinguish this from the “grids” above). This modification is not in routine use at present.
- **NO₂:** Nitrogen dioxide
- **Passive sampler/ing:** methods which absorb the pollutant directly from the ambient air, with no need of power supply.
- **TEA:** Triethanolamine. The reagent used in Palmes-type diffusion tubes as an absorbent for ambient NO₂.

4. EQUIPMENT AND MATERIALS

The nitrogen dioxide (NO₂) passive diffusion tubes will be attached to the study participants backpacks the day of the clinical examination. The tubes will be exposed for 1 week. The tubes will be collected by FW the day of the second visit at home/schools.

4.1. Equipment

- NO₂ diffusion tubs (including grids)
- Caps
- Supports for the tubes
- QR codes to identify tubes
- Laptop, the same for all monitoring instruments, with an internet access and the clock synchronized to an atomic clock
- ATHLETE ID labels
- Sealed plastic bags

4.2. Materials

- Monitoring Sheet for FW: form to be completed by the FWs when placing the tube and collecting them from the subjects' backpack
- Incidence sheet for the participants: handed to participants to report any incidences during the sampling period (in this study, is included in the diary)
- Monitoring diary for the participants or information booklet

5. PROCEDURES

5.1. GENERAL OVERVIEW

Palmes diffusion tubes are passive samplers: they consist of small glass tubes containing a chemical reagent to absorb the pollutant to be measured directly from the air. The ATHLETE study will use diffusion tubs distributed by 4Sfera, the Spanish distributor of Gradko's Laboratories. In the case of Gradko's type nitrogen dioxide diffusion tubes, the absorbent used is triethanolamine (TEA). Stainless-steel mesh grids at the closed end of the tube are coated with a water-based or acetone-based solution of this absorbent. They are useful for identifying areas of high NO₂ concentration, particularly when dealing with sources such as traffic emissions, which do not change much from day to day.

5.1.1. Diffusion process in the tubes

Diffusion tubes work by a process called molecular diffusion. During molecular diffusion, compounds will move from an area of high concentration to an area of low concentration. The compounds in the air are at a higher concentration than those in the tube, so the compounds diffuse into the tube and collect on the absorbent at the end of the tube. Because the compounds are absorbed, the low concentration in the tube is maintained, and therefore diffusion continues. The rate that the compounds move into the tube is called the uptake rate. This is a known rate and is used in the calculations during analysis. Many of the tubes are clear as light is required during the absorption process.

5.1.2. Components of the tubes

The following description is only informative, as tubes are prepared in Gradko Lab and sent to ISGlobal, so there is no need to do anything.

5.1.2.1. Tubes

Diffusion tubes should be made of clear or translucent colorless acrylic. Tubes are 7 cm tall and 1.4 cm wide. (figure1)

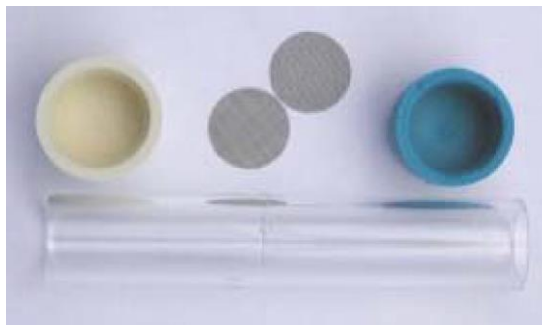


Figure 1. Diffusion Tube Components

5.1.2.2. Grids

Two grids are used per tube, and its material is stainless steel. The mesh size of this grid should be 4x4 per mm². The diameter of each circular grid should be slightly larger than the inside diameter of the tube, so that they are fixed firmly in place when the tube is assembled and cannot fall out during exposure.

5.1.2.3. End Caps

The end caps used on the end that holds the grids are opaque flexible plastic of a dark color. Sunlight can degrade the nitrite complex formed when NO₂ combines with the TEA: dark opaque caps minimize this. Colored end caps may be re-used, but must be discarded when the color begins to fade. Any cracked, discolored or split caps must be discarded. As far as possible, use end-caps of one color only in any survey: different colored caps may have different opacity, increasing the uncertainty of the results. Do not, therefore, alternate between different colors in different months. Alternating colored labels should be used instead. If possible, have spare caps in case any cap is missing when collecting the tubes.

Note: Diffusion tubes are ready-made from a supplier, so reject any that arrive with split or damaged end caps.

5.1.2.4. Supports

For ATHLETE, we will have personal clips for the tubes that will be used for personal sampling (figure 2).



Figure 2: NO₂ supports for personal monitoring

5.1.3. General instructions for the tubes

Some aspects are needed to be considered when using NO₂ diffusion tubes, in order to optimize the use of the tubes and to ensure good data quality.

5.1.3.1. Shelf life

Tubes have a shelf life of 12 weeks. Tubes must be exposed and returned for analysis within this period so they should be exposed and analyzed within 4 months of preparation. The date of reception and the expiry date (4 months after preparation, 12 weeks of shelf life) should be clearly identified for each tube batch. If preparation/components differ from the standard, the end user should be clearly notified.

Note: in case you need to extend the shelf life, NO₂tubes in particular allow an extension of its shelf life up to 16 weeks or even a bit more. Please contact the supplier for more info.

5.1.3.2. Tube storage

Tubes should be kept refrigerated before and after exposure and should not be subject to large variations in ambient temperature. Do not exceed the shelf life of 12 weeks if possible. The use of plastic containers and/or sealable clean plastic bags is essential to avoid contamination during transportation. Unexposed tubes should be stored in sealed plastic bags ideally inside a plastic container in a refrigerator.

For the blank tubes, do not remove the tube from the packaging and store them in the same conditions as the sampling tubes. If a refrigerator is not available, they should be stored in a cool dark place without temperature fluctuations.

Tubes and batches of tubes should be labeled appropriately, and enough laboratory blanks should be stored within the laboratory to measure any possible contamination during preparation. It is important that transport blanks (tubes which are not to be exposed and are left capped at all times) are included in any survey. Transport blanks should also be kept in a sealed plastic container as above. But for this study, transport blanks are not necessary.

Lab blank is the most important, then the field blank and then the transport blank.

(1) Lab blank – tube that stays in the fridge for the whole time, from the moment that the batch is received until the shipment to the lab. It is important to note the date when you receive the tubes and put them in the fridge and the shipment date when you return the tubes to the lab. (*1 lab blank for every batch received*). This blank is mandatory, for the study the procedure will be: tube arrives to ISGlobal and stays in the fridge until is sent to the cohort, the tube arrives to the cohort and stays in the fridge, the tube is sent to the lab again with the rest of the batch to be analysed. It is important to mark which tube of the batch is the blank.

(2) Field blank – tube that is placed with an exposed tube but with the cap on. Before and after being 'exposed', it goes in the fridge like any other participant tubes. (*1 field blank every 30 "exposed tubes"*). 1 participant will carry 2 tubes, 1 "exposed" tube and 1 "field blank".

5.1.3.3. Labelling the tubes with a QR code provided by the tube suppliers **(Note: this is NOT the ATHLETE ID label)**

Tubes come with an exposure sheet and a strip of QR codes; these codes are in duplicate: 1 label is for the tube and the other label is to stick it to the ATHLETE *Exposure Monitoring sheet for FW*.



Figure 4. NO₂ tubes, exposure sheets and QR codes

It is recommended that FWs label the tubes and sheets at the point of placing the tubes; this is a good way of minimizing any possible errors (figure 5). Please make sure the label is securely fixed to the tube. Be aware that these labels are prepared to deal with weather conditions, so they are heavy duty. Do not use any tubes which are cracked, damaged or have uneven ends which may prevent the end caps sealing properly. All tubes should have same dimension in terms of length and internal/external diameter.

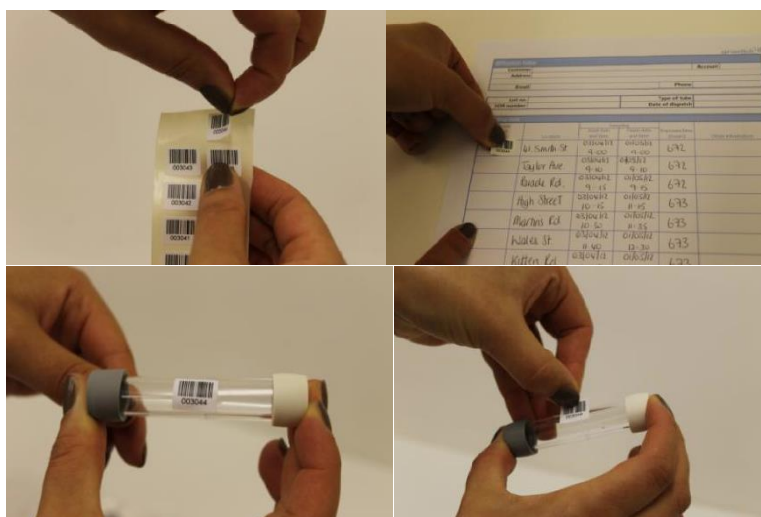


Figure 5. (from top left, clockwise) Take the 1st of the duplicate labels (1) and stick it to the appropriate location on the exposure sheet (2). Then take the 2nd matching label (3) and stick it to the tube exposed at the same location (4).

5.2. MONITORING PROCEDURE

Study subjects will carry a NO₂ tube with them during the study week to measure personal exposure to NO₂. Tubes will be placed on the outside of their backpack.

Participants need to be advised NOT to leave the backpack on the floor whenever possible but leave it on top of an elevated surface (i.e. a table, chair, etc.), ideally at the breathing height, around 1.5m or lower if sitting or sleeping. **Ask participants to avoid placing the backpack on the floor.** It is important that the open end of the tube has free circulation of air, avoiding hair, clothes, etc. blocking the entrance of the air and also not placing the tube (and the rest of the inlets) against a wall (figure 7).



Figure 7. Correct placement of the NO₂ tubes on the backpack.

Study participants will be given an incidence sheet to complete in case they have a problem or an incidence with the NO₂ tubes (“ATHLETE_FieldExposureForm_participants_incidencias” document). An incidence could be e.g. ‘tube covered with clothes for 1h’ or ‘tube moved from shoulder strap to the bottom of the backpack’. They will also be given an information booklet with information of all the devices they will have during the measurement week, so they can check in case they have doubts on how the devices work. They will be also asked to contact FWs in case they need to.

In the case the end of the sampling week does not overlap with the second visit, the participant should be asked to cap on the tube, write the day/hour in the incidence sheet and leave the tube in the refrigerator with a sealed plastic bag until collection by the fieldworkers.

5.2.1. Exposing the tubes

Remove tubes from the refrigerator on the day that they are to be used. Take the tubes to the place where the clinical examination is performed in a sealable plastic bag or plastic container. If they can be in a refrigerator, it would be preferable. But the most important is the sealed plastic bag. FWs should follow the following procedure:

1. For each participant select one tube and stick the QR code label provided by the tube supplier (**NOTE: this is NOT the ATHLETE ID label**) as you place the tubes.
2. Fill in the *ATHLETE_Exposure_Monitoring_sheet_for_FW* clearly with two labels: 1) the **ATHLETE ID label** (e.g. SAB_15y_C_0001); 2) the **QR code labels provided by the tube supplier**
3. Select the location of the tube in the backpack (the closer to the upper airways the better, ideally at the top of one shoulder strap, see Figure 7) and remove the WHITE cap. Keep the cap in a safe place (put it inside the bag that will be given to the study participants) so it can be used when the sampling week is finished. It is advisable to have extra caps in case they get lost.
4. Record the date and time of the start of the exposure period with the time of the laptop (which is synchronized with an atomic clock) on the *ATHLETE_Exposure_Monitoring_sheet_for_FW*, and complete the section for NO₂.
5. With the absorbent (colored) end cap uppermost, remove the bottom end cap (usually white or clear in color) and clip the tube into the holder of the clip. Ensure the tube is positioned vertically with its open end downwards during the required sampling period, which will be 1 week for ATHLETE (figure 7).

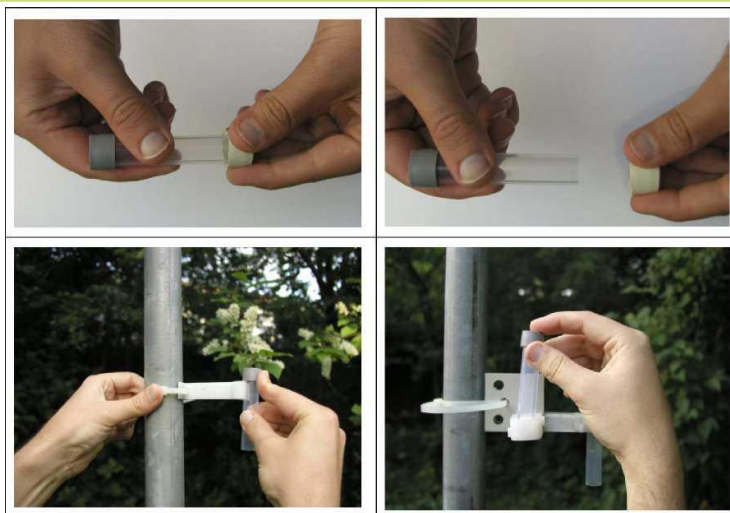


Figure 7. Removing the white cap and placing the diffusion tubes always vertically. This example is for outdoor monitoring, but provides.

5.2.2. Collecting the tubes

After the sampling period, close the tube by putting the WHITE cap back on the tube. Write the date and time down on the *ATHLETE_Exposure_Monitoring_sheet_for_FW*. Make a note of any irregularities, anything that might affect, or even invalidate the results such as insects, dirt, or liquid inside the tube. This information will be useful for the laboratory.

5.2.3. Sending the tubes for analysis

Tubes should be returned to the lab for analysis as soon as possible after exposure. However, if you store them in a refrigerator, you can collect tubes for a few weeks and send a big lot to analyze. Send also a paper copy of the *ATHLETE_Exposure_Monitoring_sheet_for_FW*, which includes exposure time information. This information will need to be sent to 4Sfera, who will send it to the lab via e-mail.

Tubes should be returned in a sealed container, such as the plastic bag that they are received in. They do not need to be sent refrigerated.

Tubes should be sent together with a Lab blank (see the definition above).

Please send the tubes to the following address and let 4Sfera know that you are sending the tubes:

Return address:

Gradko International Ltd,
St Martins House, 77 Wales Street,
Winchester, Hampshire
SO23 0RH
Tel: +44 (0) 1962 860 331
Email: diffusion@gradko.com
enquiries@gradkolab.com

Results are provided within 10 working days or can be fast-tracked by arrangement. The lab determines the concentration of compounds on the tube. This is then used in a calculation with the uptake rate to calculate the average concentration of compounds that were present in the air over the monitoring period. The results are reported in parts per billion (ppb) and micrograms per meter cubed ($\mu\text{g}\cdot\text{m}^{-3}$) to allow comparison with health guideline levels. Gradko's reports are e-mailed to ISGlobal within 10 working days of receipt of the samples. This data will be sent out by 4Sfera in axls and pdf format.

6. QUALITY CONTROL

Data will be checked to make sure the results fall within reasonable values. When tubes experience incidences, results should be carefully interpreted.

A co-location of one NO₂ tube will be done at the reference monitoring station each month during the follow-up visit, in the middle of the month for the length of one week. This will be done to validate and calibrate the measurements comparing the results to those from the Government Reference Fixed Stations.

Internal quality of the tubes will be also check by having 2 laboratory blanks each time tubes are sent to the Lab in the UK. These tubes will be kept in the fridge properly sealed in plastic bags, along with the non exposed tubes.

7. REFERENCES

- *Nitrogen dioxide technical data sheet by Gradko.*
- *Diffusion Tubes for Ambient NO₂ Monitoring: Practical Guidance for Laboratories and Users Report to Defra and the Devolved Administrations. ED48673043. Issue 1a. Feb 2008. AEA Energy & Environment.*
- *Long-Term Exposure to Urban Air Pollution and Mortality in a Cohort of More than a Million Adults in Rome. Mapping for change. Air quality mapping Toolkit. NO₂. 2015*
- *Gradko's Technical Data Sheet*
- *Study protocol air pollution exposure assessment for the REGICOR – Air Study population*

Anotar en la hoja de monitorización (ver apartado formularios de registro) el código QR de la etiqueta para el tubo, la fecha del día 0 de inicio y la del día 8 de recogida

Entregar a los voluntarios las instrucciones en el Diario semanal de monitorización (ver documento Anexo5c_Diario_semanal_ATHLETE)

Col-laboradors:



Finançadors:

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Omega-Brain

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